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Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

F667X

From Page No. \_\_\_\_\_

Spin down 1.2L cells - GS-5 - 7000 RPM - 30 min - Decant -  
- Dissolve 86 grams cells in 25ml Crack BFR (pg 7)

centrifuge 4 x 3.5 30 sec. (t) bat 4.5 30 sec each

A 590 1.200 d/n

Crack - .98  $\approx$  70% crack  $\rightarrow$  Heat 15 min @ 88°C.  
Female - .13 Cool 10 min / ice

ADD 0.4% PET (1.2 ml 10% stock) - STIR 15 min

Spin in SS-34 9K - 30 min - Decant 50%

7.55 gms  $\rightarrow$  ADD NH<sub>4</sub>SO<sub>4</sub> - 308 g/L (40% cut) - STIR 4°C 45 min

- Spin down in SS 34 12K - Decant sup, save for H<sub>2</sub>O  
Resuspend pellet in 8 ml BFR A - dialyze into 4°C 15.50 ml

BFR A

25 mM Tris - 7.4

8% glycerol

0.5 mM EDTA

10 mM KCl

5 mM Bme - (1.3 ms)

BFR B (High salt gradient)

Same as BFR A

TOSO-650 Heparin

Down Sul column Equilibrate w/ A

Load at 0.5 ml/min

wash w/ B UTs - until baseline at 1.5 ml/min

Change dialysis buffer once -

Bump 4 ml TOSO 650 Heparin - 4M Gu HCl - 3M NaCl 10 vts

Wash with H<sub>2</sub>O -

Equilibrate w/ BFR A - CRAD = (1.4 ms) CRAD = 7.1 ml -

Load - 75 ml/min

10 vts gradient A  $\rightarrow$  B - same for both

Mix - premade by H.G. - stored @ -20°C - same rxn mix as for native

Witness d & Understood by me,



Date

2/27/95

Invented by



Date

12-5-95

Recorded by

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